

# THE ROLE OF COMPETITIVE INTERACTIONS, PHENOLICS, AND FEEDING BY MESOGRAZERS IN EURASIAN WATERMILFOIL (*MYRIOPHYLLUM SPICATUM*) INVASIONS

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## Abstract

Invasive plants can spread to a degree that disrupts the structure of an ecosystem and causes damage to the environment. Factors contributing to plant invasiveness are incompletely understood, but elevated levels of chemical deterrents may enhance invasion success. This study examined competitive interactions, chemical defense production, and palatability in native *Ceratophyllum demersum*(coontail) and invasive *Myriophyllum spicatum* (milfoil). A laboratory competition experiment examined differences in *C. demersum* and *M. spicatum* growth in monoculture and polyculture. Since coontail can allelopathically reduce the growth of some plants, *M. spicatum* was grown in water containing chemical cues from each species. Field-collected samples of each species were freeze dried and ground for phenolic analysis and a palatability experiment. Freeze-dried, ground tissue from each plant species was incorporated into an artificial diet that was offered to amphipods (*Gammarus* sp.) in a choice feeding experiment. Coontail grew similarly well in both monoculture and polyculture, while milfoil grew better in polyculture. Milfoil growth was lowest in milfoil-conditioned water that contained its own chemical cues, showing that coontail did not allelopathically inhibit the growth of milfoil. Phenolic analysis showed that milfoil contained more phenolics than coontail, which should lead to greater amphipod feeding on the agar food made with coontail than with milfoil. Although other factors could affect competitive interactions and plant palatability, the results suggest that coontail can successfully compete with milfoil and that herbivores may alter competitive interactions between these species.

## Introduction

*Myriophyllum spicatum* (Eurasian watermilfoil, hereafter known as milfoil) is highly successful in colonizing new habitats and can outcompete and replace other submersed macrophytes (Grace and Wetzel 1978). Some studies have suggested that competition may influence aquatic plant invasion success (Zhu and Georgian 2014), but it is unclear how plant competitors affect the growth of invasive plants. *Ceratophyllum demersum* (coontail) is a common native plant in areas colonized by milfoil. Coontail is known to allelopathically inhibit the growth of other species (Gross et al. 2003), so its chemical exudates may reduce milfoil growth. In addition to competition, herbivores can also contribute to successful plant invasions. Milfoil produces high levels of chemically deterrent phenolics that make it unpalatable to some aquatic herbivores (Fornoff and Gross 2014). If native plants produce lower levels of chemical deterrents like phenolics, those native species may suffer greater tissue losses to herbivory than more chemically defended invaders like milfoil. To foster a more complete understanding of the factors that make milfoil such a successful invader, this study examined the competitive interactions, chemical defense production, and palatability of native *Ceratophyllum demersum* and invasive *Myriophyllum spicatum*.

## Hypotheses

1. Coontail growth is lower when grown with milfoil than when grown alone.
2. Allelopathic chemicals from coontail reduce milfoil growth.
3. Milfoil has higher chemical deterrent levels (phenolics) than coontail.
4. Amphipods prefer coontail to milfoil when exposed to both simultaneously.

## Methods

### Plant Collection & Preparation

- Native coontail and invasive milfoil were collected from Osbourndale Pond in Derby, CT for use in lab competition and allelopathy experiments
- Plants were held in 7-L tanks containing bubbled tap water under ambient light prior to use in experiments
- 10-ml tubes were filled with whole stems of either milfoil or coontail (n = 25 per species) and flash frozen at -80°C, then freeze dried and ground for phenolic analysis and use in feeding trials

### Phenolic Analysis

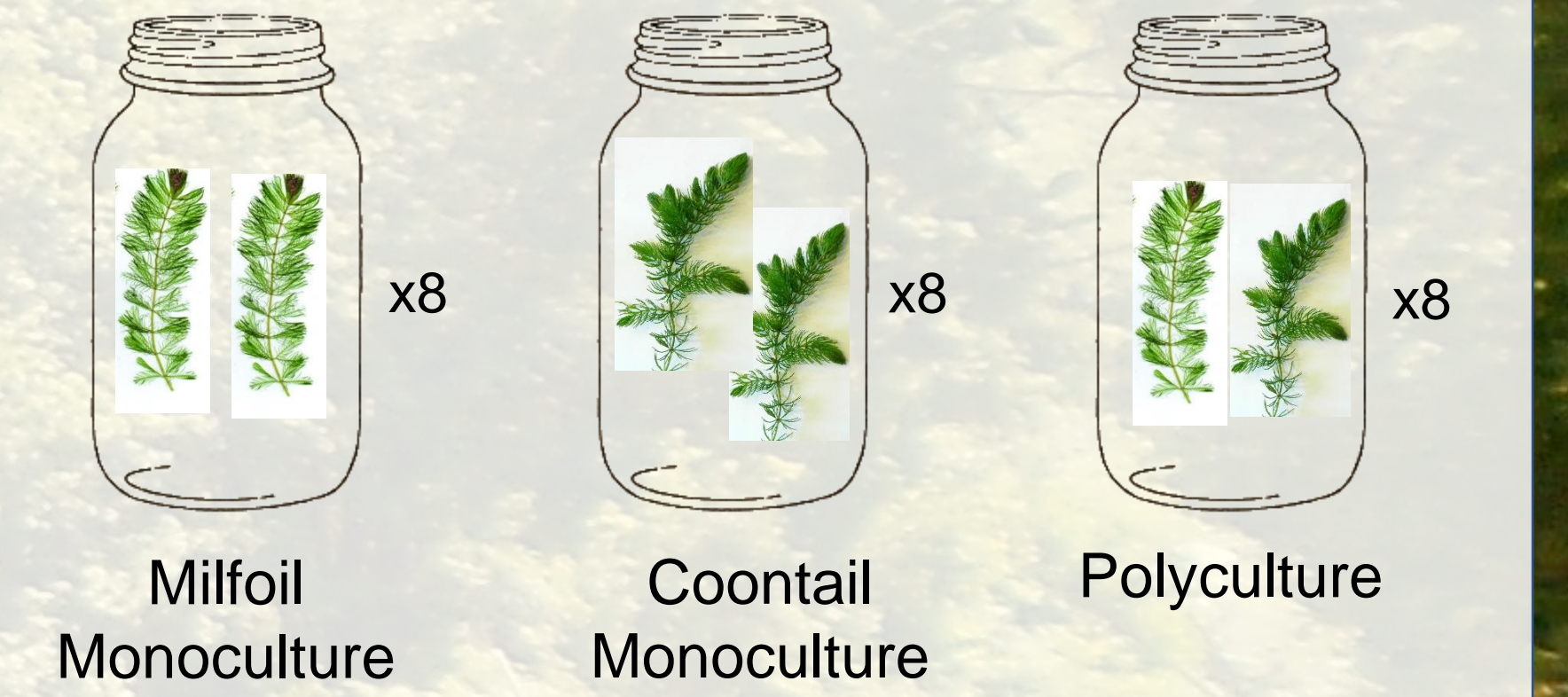
- 1 mM ascorbic acid in 70% acetone was used to extract phenolics from freeze-dried, ground milfoil and coontail samples (n = 7 milfoil, n = 8 coontail)
- Total reactive phenolics were measured using the colorimetric Folin-Denis assay

### Choice Feeding Experiment

- Amphipods (*Gammarus* sp.) were added to 500-ml bowls containing ~250 ml spring water (10 amphipods per bowl, n = 10)
- Artificial diets of 0.3 g agar, 20 ml deionized water, and 0.5 g of either milfoil or coontail tissue were spread across squares of fiberglass screen (1 mm mesh size)
- One square of each diet (milfoil and coontail) was placed into each bowl with amphipods, and the number of squares cleared of food was recorded after six days
- Five autogenic controls contained only spring water and each agar diet

### Competition Experiment

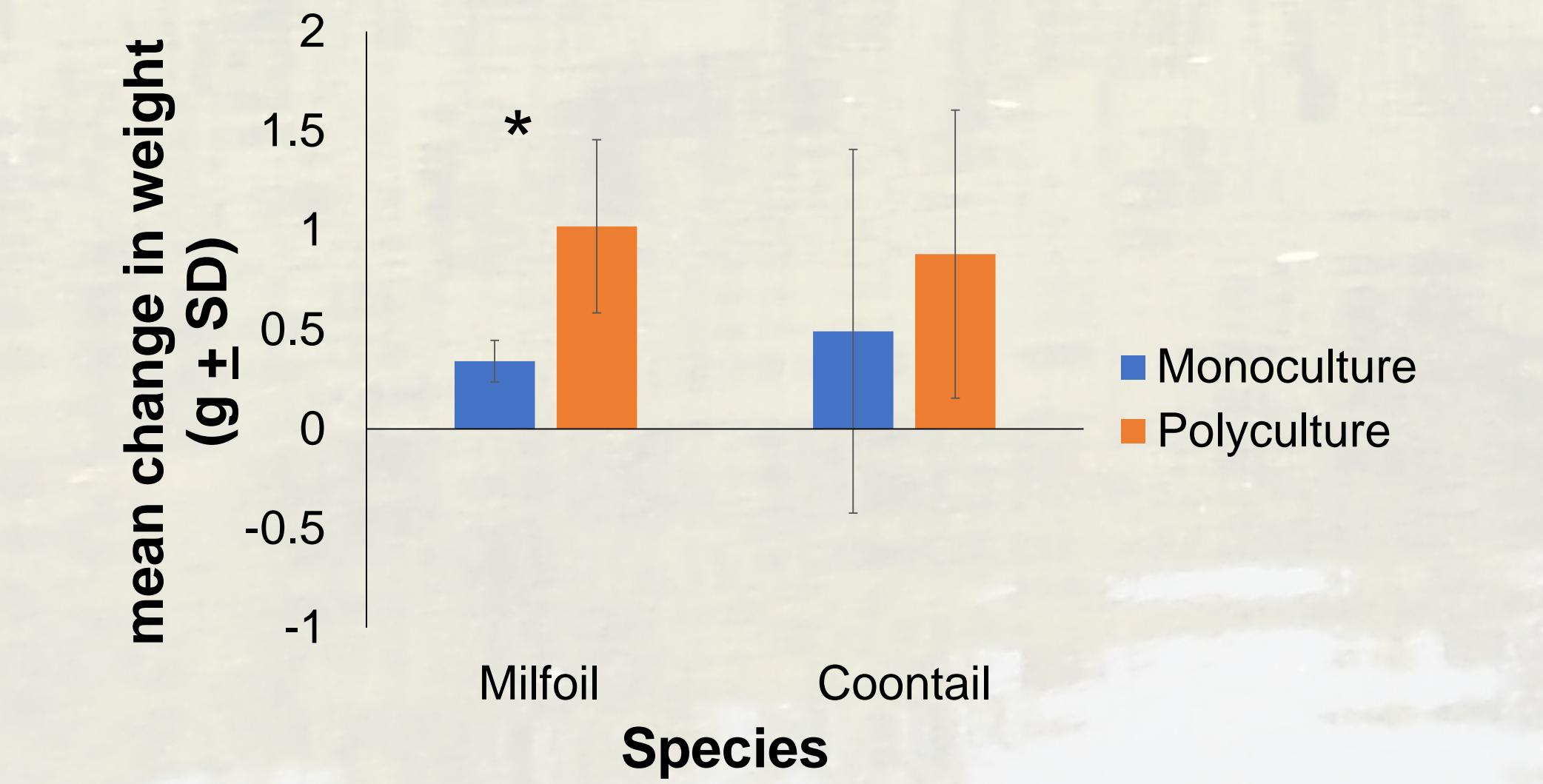
- Eight replicates of three treatments (diagram below) were set up in 1-L glass jars containing bubbled tap water and an air stone under grow lights on a 12 hour light: 12 hour dark cycle
- 10-cm plant fragments were weighed before being placed in jars and again after two weeks; plants were frozen for later phenolic analysis
- Water containing chemical cues from each species was immediately re-used in the allelopathy experiment



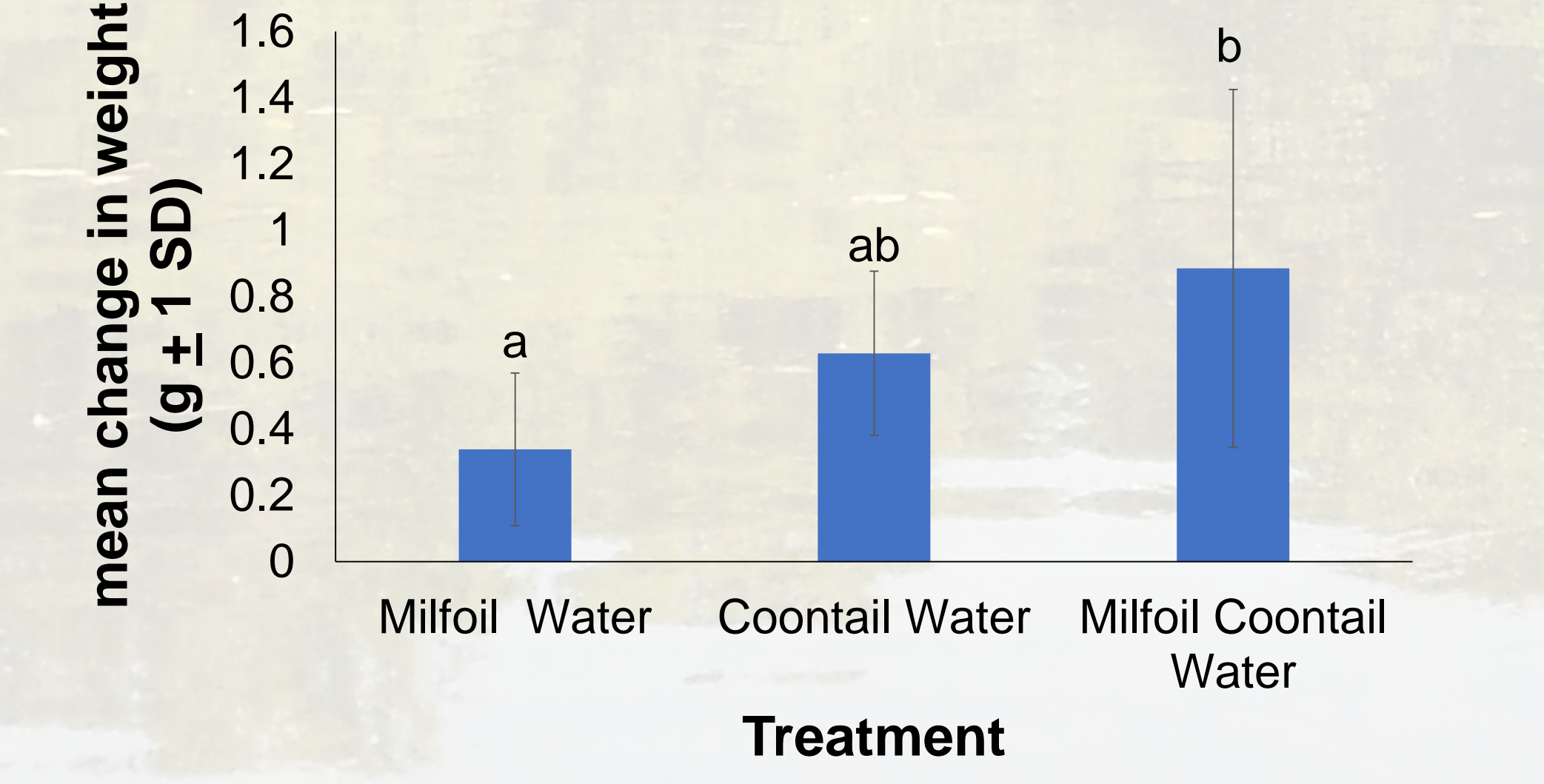
### Allelopathy Experiment

- One pre-weighed 10-cm milfoil fragment was placed into each jar from the competition experiment (above)
- Fragments were re-weighed after ten days

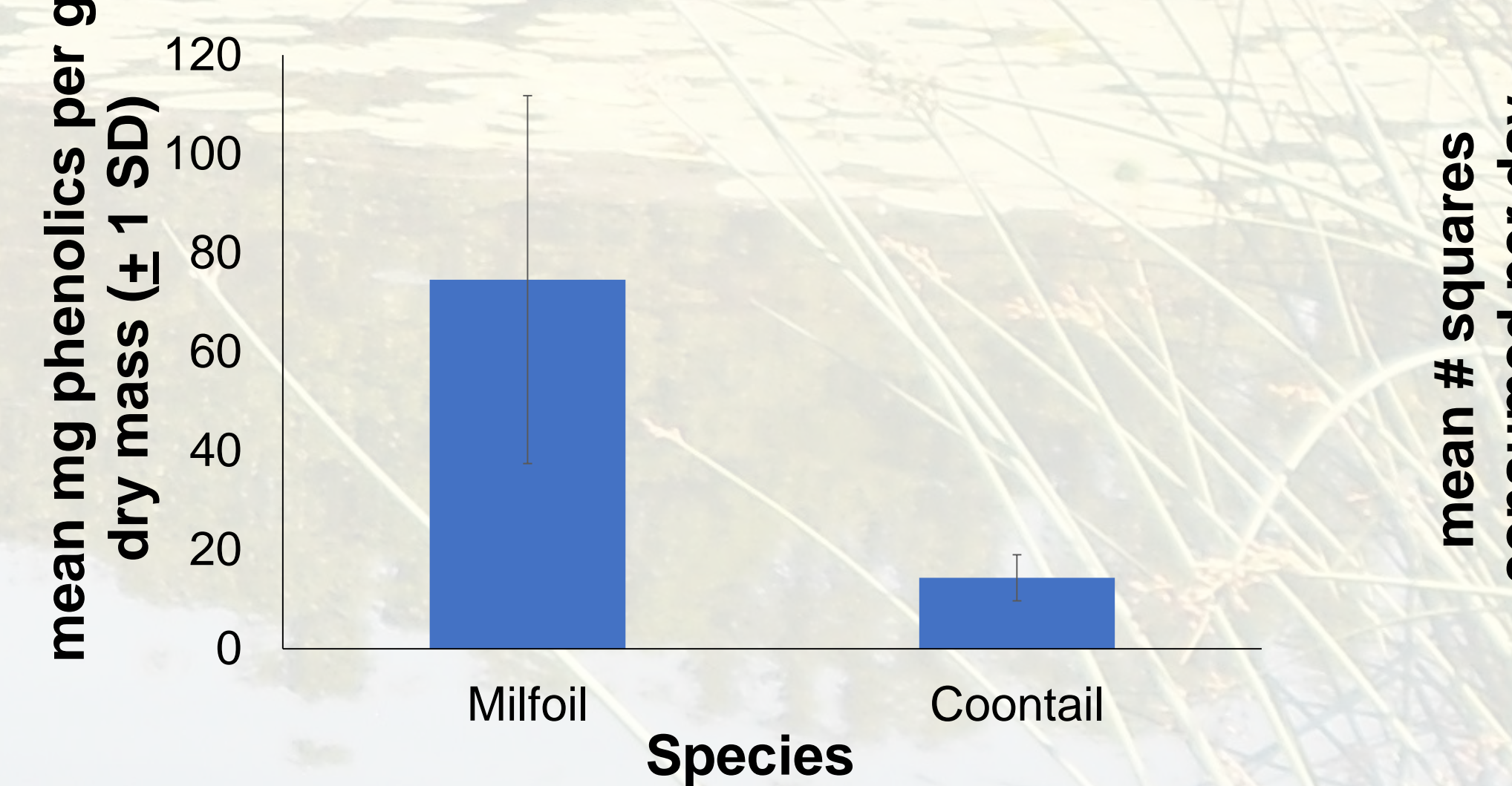
## Results



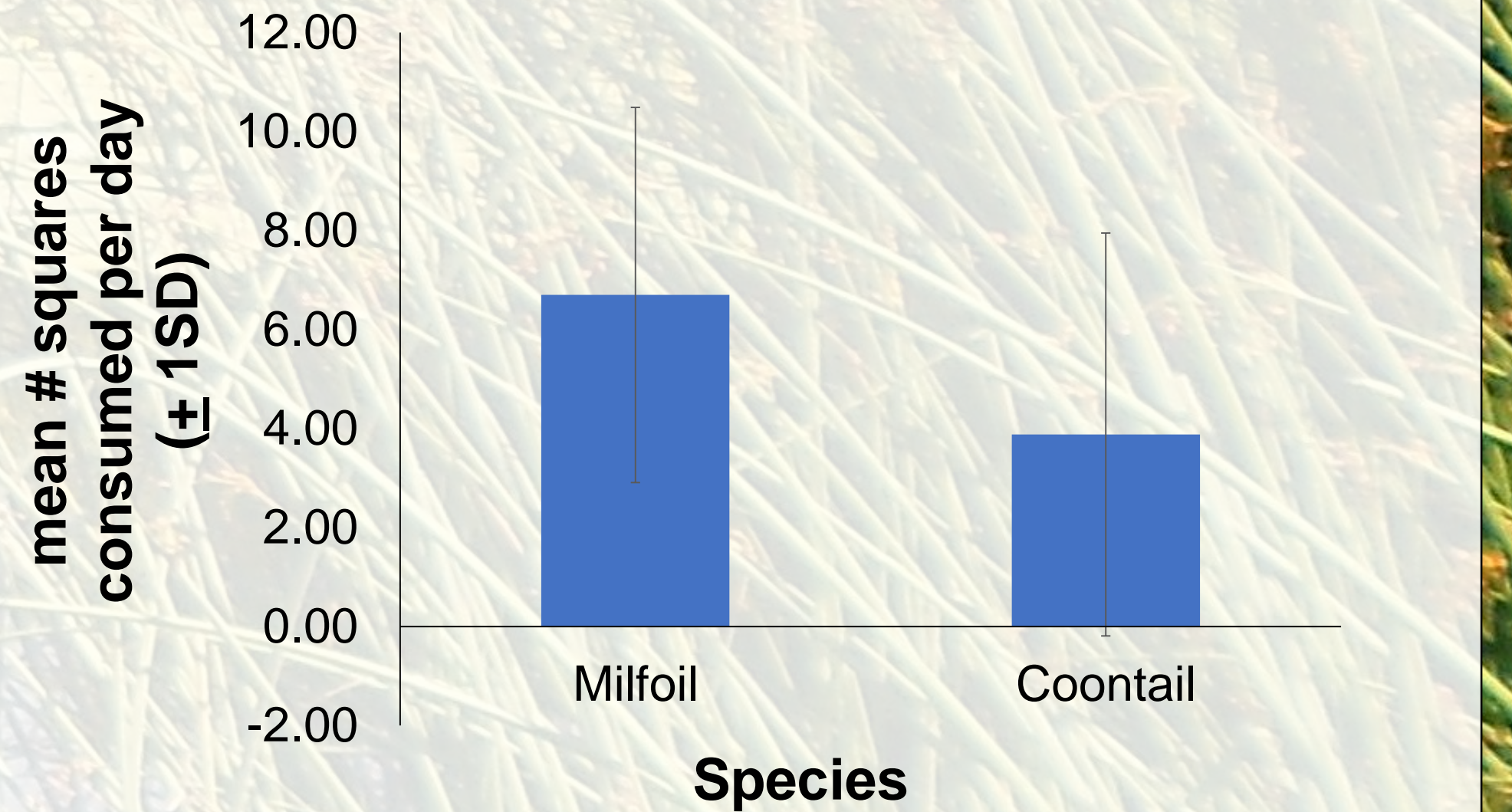
**Figure 1.** Mean change in weight ( $g \pm 1$  SD) of invasive *Myriophyllum spicatum* (milfoil) and native *Ceratophyllum demersum* (coontail) fragments after 14 days in a competition experiment. **Milfoil grew significantly better in polyculture than in monoculture** (independent samples t-test:  $t=-4.288$ ,  $p=0.003$ ). **Coontail growth was similar in monoculture and polyculture treatments** (independent samples t-test:  $t=-0.941$ ,  $p=0.36$ ).



**Figure 2.** Mean change in weight ( $g \pm 1$  SD) of invasive *Myriophyllum spicatum* fragments after growing in water containing chemical cues from itself (Milfoil Water), native *Ceratophyllum demersum* (Coontail Water), or both milfoil and coontail (Milfoil Coontail Water) for 10 days in an allelopathy experiment. **Invasive milfoil grew significantly less when grown in water containing chemical cues from itself than when grown in water with cues from both species** (ANOVA:  $F_{2,21}=4.865$ ,  $p=0.018$ ). Different letters over the error bars indicate significant differences between treatments in post-hoc Tukey pairwise comparisons.



**Figure 3.** Mean mg of phenolics per gram of dry mass ( $\pm 1$  SD) in invasive *Myriophyllum spicatum* (milfoil) and native *Ceratophyllum demersum* (coontail). **Phenolic production was significantly higher in invasive milfoil than in native coontail** (independent samples t-test:  $t=4.567$ ,  $p=0.001$ ).



**Figure 4.** Mean squares of agar food made with invasive *Myriophyllum spicatum* (milfoil) and native *Ceratophyllum demersum* (coontail) tissue consumed per day by amphipods (*Gammarus* sp.) after 6 days in a choice feeding experiment. Agar loss in autogenic controls was subtracted from the raw consumption values. **Amphipods showed no significant preference for either species** (paired t-test:  $t=1.544$ ,  $p=0.157$ ).

## Conclusions

**Competition and Allelopathy:** Our results showed no evidence that invasive milfoil reduced the growth of native coontail, although milfoil grew more in polyculture than in monoculture (Fig. 1). This suggests that coontail's presence increases milfoil's growth rate without suffering declines in its own growth. Coontail did not allelopathically inhibit the growth of invasive milfoil (Fig. 2), contrary to our hypothesis. However, milfoil's increased success in the presence of coontail (Fig. 1) may have resulted from coontail reducing the growth of microalgae in the water (Gross et al. 2003).

**Phenolics and Palatability:** Unsurprisingly, milfoil contained almost four times more phenolics per gram of tissue than did coontail (Fig. 3). However, this difference in phenolics did not translate to a reduction in milfoil's palatability compared to coontail (Fig. 4). Our experimental design eliminated structural differences between milfoil and coontail that may contribute to their palatability. Previous data showed that amphipods consumed more coontail than milfoil when offered whole plant tissues (Steele, unpublished data). Our new data indicate that those differences in palatability were not driven by chemical differences between the two plants (Figs. 3 & 4). However, it is still possible that milfoil's high phenolic levels affect its palatability compared to other aquatic plant species (Fornoff and Gross 2014). Overall, our results suggest that the presence of native coontail may prevent milfoil from overgrowing waters colonized by the non-native.

## Literature Cited

Fornoff F, Gross EM. 2014. Oecologia 175:173-185.  
Grace J, Wetzel R. 1978. J. Aquat. Plant Manage. 16: 1-11.  
Gross E, Erhard D., Ivanyi E. 2003. Hydrobiologia 506: 583-589.  
Zhu B, Georgian SE. 2014. Plant Ecology 7(6):499-508.

## Acknowledgements

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